Remarks:

The amendments to claims 3, 5-8, 11, 25, 26, 29, 31-34, 37, and 39-40 as submitted in the amendment filed on February 24, 2005, were not entered for the reasons stated in the Advisory Action mailed on April 19, 2005. As such, amendments made to those claims on August 22, 2005, were likewise not entered. Applicant has submitted an amended claim set based on claims that were pending as of February 24, 2005.

I. Amendment to the claims

Independent claims 1 and 3 were amended to include, and newly added claim 41 includes, the limitations that:

(a) the cell line of the claim has been transformed with a first expression vector comprising a coding sequence for an anti-apoptotic protein operably linked to a first promoter, and a second expression vector comprising the coding sequence for PKR operably linked to a second promoter; and

(b) the transformed cell line being characterized by a level of interferon-alpha production that is significantly greater than the level of a control cell line transformed with PKR alone when the transformed cell line and the control line are grown under cell culture conditions of interferon-alpha production induced by the addition of Sendai virus.

Support for limitation (a) is found, for example, in originally presented claim 5, when considered with the limitations of originally presented claim 3, and more generally, in Section VII on page 25-29 and in Example II on pages 35-36. Support for limitation (b) is found, for example, on page 37, line 13-16.

No new matter has been added by these amendments.

II. Rejections under 35 U.S.C. §103(a)

Claims 1-3, 5-8, 11, 25, 26, 29, 31-34, 37, 39, and 40 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Dixit (U.S. Patent No. 6,159,712), Lau et al., (U.S. Patent No. 6,159,712) and Suzuki et al. (Derwent Abstract XP-02170158). This rejection is respectfully traversed.

A. The invention

The present invention, as embodied in claim 1, is directed to a human cell line for use in producing one or more cytokines. The cell line has been transformed to express both a gene for PKR and a gene for an anti-apoptotic protein, and it is capable of producing levels of cytokine in culture, e.g., interferon-alpha, that are several fold greater than that produced by an untransformed cell line or a cell line transformed with the PKR gene alone.

The problem addressed by the invention is the need for methods of further enhancing cytokine production in a mammalian cell line **that has been transformed to over-express PKR and shows a reduced viability in culture, presumably due to elevated levels of produced cytokines.**

B. The cited prior art

Lau describes a method to increase production of interferon in an animal cell line by inducing the cell line to over-express PKR. Lau inherently discloses the problem faced by the applicants but in no way suggests a solution to the problem.

Dixit describes a method for preventing or inhibiting apoptosis in a cell. The method includes introducing a nucleic acid coding for CrmA or a nucleic acid coding for a gene product having CrmA biological activity. Dixit teaches compositions and methods for maintaining the viability of T cells in an HIV-infected individual. (column 1, lines 65-66 and column 2, lines 1-15). Dixit is not concerned with enhancing levels of cytokine production, and in particular, Dixit is not concerned with enhancing levels of cytokine production in PKR overproducing cells.

Suzuki et al. describe a method of improving production of a useful target material, such as antibodies, cytokines, etc., from a cell by inhibiting apoptosis of the cell by introducing into the cell an apoptosis inhibitor gene. Like the Dixit reference above, Suzuki does not recognize the problem of enhancing cytokine production in PKR over-expressing cells, nor does the reference suggest a solution to the problem, for the reasons discussed below.

C. Analysis

A *prima facie* case of obviousness under 35 U.S.C. §103(a) requires the following elements:

> (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify or combine the references.
>
> (2) there must be a reasonable expectation of success.
>
> (3) the prior art references when combined must teach or suggest all of the claim limitations. M.P.E.P. § 2143.

In addition, a claimed invention cannot be considered obvious if the cited art, taken either individually of in combination, fails to suggest the advantages achieved by the invention.

C1. No suggestion or motivation to combine.

The problem addressed by the applicants' invention is the need for further enhancing cytokine production in cells which:

> (1) have a stimulated production of cytokines by virtue of over-expression of PKR; and
>
> (2) show reduced viability in culture, presumably due to elevated levels of produced cytokines.

This is a problem that is inherent in Lau, however, Lau does not recognize the problem nor offer any suggestion as to its solution.

One skilled in the art would not be motivated to look to Dixit for a solution to this problem, since Dixit does not teach or suggest (i) cytokine production in cells, or (ii) the effect of enhanced cytokine production on cell viability. Dixit therefore fails to address the problem of enhancing cytokine production in PKR-overexpressing cells cultured under conditions of cytokine induction.

Similarly, one would not look to Suzuki for a solution to this problem. Suzuki discloses that an anti-apoptotic gene can be used for enhancing cellular production, and teaches that the method may be applicable for the production of a variety of proteins, including antibodies, vaccine, cell growth factors, and cytokines. Suzuki never actually produced cytokines, but rather, only showed increases in the speed (~2x) and quantity (~4x) of antibody production due to the introduction of an anti-apoptotic gene. Most importantly, Suzuki never addressed the problem solved by the present invention – the need for methods of further enhancing cytokine production in a mammalian cell line that has been transformed to over-express PKR and shows a reduced viability in culture, presumably due to elevated levels of produced cytokines. One skilled in the art that is attempting to solve this problem would have little to no reason to believe that the methods taught Suzuki for antibody production would even be applicable in such a mammalian cell line.

C2. No reasonable expectation of success:

At least for the reasons stated above, the applicant respectfully submits that the examiner is incorrect in the assertion that one of skill would have a reasonable expectation of success when combining Suzuki's anti-apoptotic gene expression in a cell with Lau's over-expression of PKR.

The legal standard is not whether the prior art makes it "obvious to experiment" to arrive at the invention - impermissible hindsight is no more applicable to designing experiments to arrive at the invention than it is to combining the prior art teachings to arrive at the invention. See In re Dow, 5 USPQ2d 1529,

1532 (Fed. Cir. 1988). A reasonable expectation of success must be found in the prior art.[1] See Id. at 1531.

The desirability of inhibiting apoptotic cell death in a PKR-expressing cell line to prolong the lifespan of the cells during cytokine induction was stated by the applicant at page 4, lines 40-42 of the specification. And, it is true that the state-of-the art shows knowledge of the use of anti-apoptosis genes and PKR over-expression. But, as the court pointed out in In re Dow, the fact that individual methods may each provide a given result when used separately does not necessarily mean that one of skill could reasonably expect that a combination of the methods will work together to provide a desired combination of results. This is particularly true in unpredictable and complex arts, such as the field of the present invention.

First, the initial discovery that PKR over-expression would enhance cytokine production was unexpected in the art and is a reminder that this is an unpredictable art. The applicant teaches, at page 12, line 36, through page 13, line 22, of the specification, that PKR activity is complex and is involved with at least (1) signal transduction for complex receptor systems (including IFN, TNF, and Fas); (2) transcriptional activation of cytokine genes; (3) initiation of apoptosis; and (4) inhibition of protein synthesis. These points were discussed in great detail in the amendment filed on April 21, 2004, and were further supported in that amendment

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[1] The claims at issue in In re Dow recited an impact resistant rubber-based resin that is suitable for molding and extrusion, and has an improved resistance to heat distortion, and the resin is a reaction product of (1) styrene, (2) maleic anhydride, and (3) synthetic diene rubbers. Two references were cited against the claims: the first reference teaches how to make an impact resistance rubber by dissolving a synthetic diene rubber in styrene and polymerizing the styrene; and the second reference teaches how to make a styrene-maleic anhydride copolymer. The Patent Office held that the claimed rubber was obvious, alleging that one of skill could have combined the references to produce the claimed rubber having the desired properties. The Federal Circuit held that, viewed in light of the prior art, the references did not provide a reasonable expectation of success in combining the references to produce the claimed rubber having a three-component system, since there were reasons to be skeptical about the success of combining the components to produce the desired results.

by citing to, and submitting to the examiner, several pieces of scientific literature. It was unexpected that a protein synthesis inhibitor and an inducer of apoptosis would enhance the production of cytokines.

There is simply no reasonable expectation that the two different transfection mechanisms, each capable of causing elevated cytokine production in cultured cells, would provide the desired results. Given the complexity of cytokine production and its feedback on cells, there was a low predictability of succeeding with the double transfection of the present invention. Since PKR is known to be integral to the initiation of apoptosis and inhibition of protein synthesis, it is counterintuitive that over-expression of PKR would result in a greater level of cytokine production. And, furthermore, given the state-of-the-art, one of skill would have actually expected the problems associated with cytokine overproduction in PKR over-producing cells to be aggravated by the claimed combination of transfections. There is nothing in the prior art that suggests otherwise, which leads to the conclusion that the hindsight reconstruction of the teachings of the specification was used as a basis for this obviousness rejection. In fact, the examiner even made the following admission at page 7 of the office action dated October 21, 2003:

> "It is well established in the art that the apoptosis induction has many stimuli, including the induction by cytokines. In an effort to express cytokines from a cell the production and release of the cytokine into the medium/supernatant will have a negative effect on the cell producing the cytokine. This is a negative feedback loop [that] normally would function to turn off cytokine production, however, cells that keep producing cytokine will eventually die because the effect is to trigger the apoptotic event. Once too much cytokine has been produced the cell is then stimulated to begin the path of self destruction."

This admission by the examiner supports a conclusion of nonobviousness of the claims and supports the conclusion that, at best, an experiment that includes the claimed combination of transfections may merely be obvious to try, which as stated above, is not the standard for obviousness under 35 U.S.C. § 103.

C3. No suggestion of the advantages obtained by the combination.

None of the cited references, taken individually or together, suggest the advantages achieved by the invention. The applicant respectfully submits that the examiner is incorrect in the assertion that the data provided by the specification indicates that the combination of transfections for the expression of Bcl-X1 and PKR in a single cell line merely produces additive results.

The examiner points to the data provided in Figs. 1 and 2 of the present application, however, Figs. 1 and 2 show only a single transfection – a transfection with only an anti-apoptosis gene. No conclusions can be drawn about the combined effect of the double transfection of the invention using the data provided in Figs. 1 and 2. The examiner then attempts to use the data provided in Figs. 4 and 5 to try and support a conclusion that the results are merely additive - the applicant fails to understand this analysis. The examiner states that the data shows an expected additivity, whereas the data clearly shows a lack of additivity. The data exemplifies an unpredictability in results that was shown to exist, for example, among the choices of methods used to induce cytokine expression under conditions of PKR overproduction.

Claim 1 recites the limitation of "said cell line being characterized by a level of interferon-alpha production that is significantly greater than the level of a control cell line transformed with PKR alone when the transformed cell line and the control line are grown under cell culture conditions of interferon-alpha production induced by the addition of Sendai virus." In order to conclude that adding an anti-apoptotic gene to PKR over-expressing cell line would provide an additive result, the comparison should be made between (i) the IFN production using a single transfection with a PKR gene and (ii) the IFN production using the double transfection with the PKR gene and an anti-apoptotic gene, both under conditions of induction with the Sendai virus. Figs. 4a and 5a clearly show that the cell viability increase with the addition of the anti-apoptotic gene under these conditions was about 34%, whereas the increase in productivity with the addition of the anti-

apoptotic gene was about 700%. These results are clearly not additive under this set of conditions and are clearly synergistic.

The examiner has made an admission on the record that one of skill would expect the results of the double transfection, the resulting cytokine production, to be additive. As noted above, and included as a claim limitation in all of the pending claims, the transformation of cells with expression vectors for both PKR and an anti-apoptotic protein significantly enhances the level of cytokine obtained in culture relative to PKR-transformed cells alone when induced by Sendai virus. The results show a several fold increase in interferon-alpha production over PKR-overproducing cells. None of the references, alone or in combination, demonstrates any such increase in any cytokine production by transforming PKR over-expressing cells with an anti-apoptotic gene and, as such, simply do not suggest the advantages obtained by the present invention.

In view of the above arguments, the currently amended claims cannot be considered obvious over a combination of Lau, Dixit, and/or Suzuki. Accordingly, for at least the reasons provided above, withdrawal of the rejection under 35 U.S.C. § 103 is respectfully requested.

III. Conclusion

This amendment shows that there was no suggestion or motivation to combine, no reasonable expectation of success, nor any suggestion of the advantages obtained by the invention in the prior art. And, Applicant has shown that the results obtained with respect to the claimed invention were synergistic. In view of the foregoing, Applicant submits that the claims pending in the application are in condition for allowance. A Notice of Allowance is therefore respectfully requested.

The undersigned invites the Examiner to call (650) 838-4388 with any questions or comments. The Commissioner is hereby authorized and requested to charge any deficiency in fees herein as needed to allow entry and consideration of this amendment to Deposit Account No. 50-2207.

Respectfully submitted,

Perkins Coie LLP

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